Pesticide Biochemistry and Physiology xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Pesticide Biochemistry and Physiology

journal homepage: www.elsevier.com/locate/pest

Mini review: Mode of action of mosquito repellents

Joseph C. Dickens *, Jonathan D. Bohbot

United States Department of Agriculture, Agricultural Research Service, Henry A. Wallace Beltsville Agricultural Research Center, Plant Sciences Institute, Invasive Insect Biocontrol and Behavior Laboratory, Beltsville, MD, USA

ARTICLE INFO

Article history:
Available online xxxx

Keywords:
Insect repellent
Feeding deterrent
Olfaction
Gustation
Modulation

ABSTRACT

The mode of action of mosquito repellents remains a controversial topic. However, electrophysiological studies and molecular approaches have provided a better understanding of how repellents exert their effects. Here, we briefly discuss various theories of repellent action and present the current status of knowledge of the effects of repellents on olfactory and gustatory processes. These findings provide a framework for further development of existing repellents and the discovery of new compounds with novel modes of action.

Published by Elsevier Inc.

1. Introduction

Mosquitoes vector numerous diseases including malaria, dengue, west nile virus and yellow fever. Even in the absence of disease, mosquitoes are an annoyance that can disrupt outdoor activities. The use of repellents decreases contacts between mosquitoes and their hosts, and may even lower the rate of disease transmission in many instances [1]. The most commonly used mosquito repellent, DEET (*N*,*N*-diethyl-3-methylbenzamide), was discovered over 60 years ago and has been in use since the 1950's [2]. Many other compounds have been characterized as having repellent activity for mosquitoes as well as other arthropod vectors based on laboratory behavioral bioassays or topical application of the compounds to the skin for field and laboratory testing [3].

Here, we briefly outline various theories on the mode of action of repellents. Then we present recent studies mostly from our lab, which provide insight into some of the early theories on the mode of action of insect repellents, and a model for future research aimed at discovery of new compounds with repellent action.

2. Theories of repellent action

2.1. DEET masks responses of olfactory receptor neurons (ORNs) to attractants

The first detailed investigations of the mode of action of repellents were summarized by Davis [4]. At the time, techniques were available for single cell recordings from ORNs on the antennae of mosquitoes and a number of repellent compounds were tested for their activity on these cells. Based mostly on these electrophysiological studies, Davis and his colleagues hypothesized that repellents had their effect by modifying or blocking responses of ORNs normally sensitive to attractants. This idea was supported by the observation that DEET decreased the sensitivity of both lactic acid sensitive ORNs to lactic acid, a component of human sweat [5], and an ORN sensitive to an oviposition attractant, ethyl proprionate [6].

2.2. DEET exerts its effects by activating specific ORNs or specific odorant receptors (ORs)

Boeckh and his colleagues [7] showed that two ORNs (based on different action potential amplitudes) associated with A-2 sensilla on the antenna of *Aedes aegypti* were activated by DEET. They postulated that since these neurons were not activated by attractants that a message may be sent to the central nervous system which counteracts the perception of attractants by other neurons. However, they did not rule out direct inhibition of an attractant receptor neuron as Davis and his colleagues had shown earlier [5,6]. Syed and Leal (2008) showed that DEET activated a specific ORN in a trichoid sensillum on the antennae of *Culex quinquefasciatus* [8]. The demonstration that DEET activated a specific odorant receptor (OR) in larval *Anopheles gambiae* provided additional support for this theory [9].

2.3. DEET sequesters an attractant

Syed and Leal [8] showed that when DEET was released from odor cartridges with the attractant component, octenol, the

* Corresponding author. Address: USDA, ARS, BARC, PSI, IIBBL, 10300 Baltimore Avenue, Beltsville, MD 20705, USA. Fax: +1 301 505 6580.
E-mail addresses: jdickdickens@comcast.net, joseph.dickens@ars.usda.gov (J.C. Dickens).

0048-3575/$ - see front matter Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.pestbp.2013.02.006

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
2013		**00-00-2013 to 00-00-2013**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Mini review: Mode of action of mosquito repellents	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **United States Department of Agriculture, Agricultural Research Service,Henry A. Wallace Beltsville Agricultural Research Center,10300 Baltimore Avenue,Beltsville,MD,20705**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The mode of action of mosquito repellents remains a controversial topic. However, electrophysiological studies and molecular approaches have provided a better understanding of how repellents exert their effects. Here, we briefly discuss various theories of repellent action and present the current status of knowledge of the effects of repellents on olfactory and gustatory processes. These findings provide a framework for further development of existing repellents and the discovery of new compounds with novel modes of action.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**Same as Report (SAR)**	**7**	

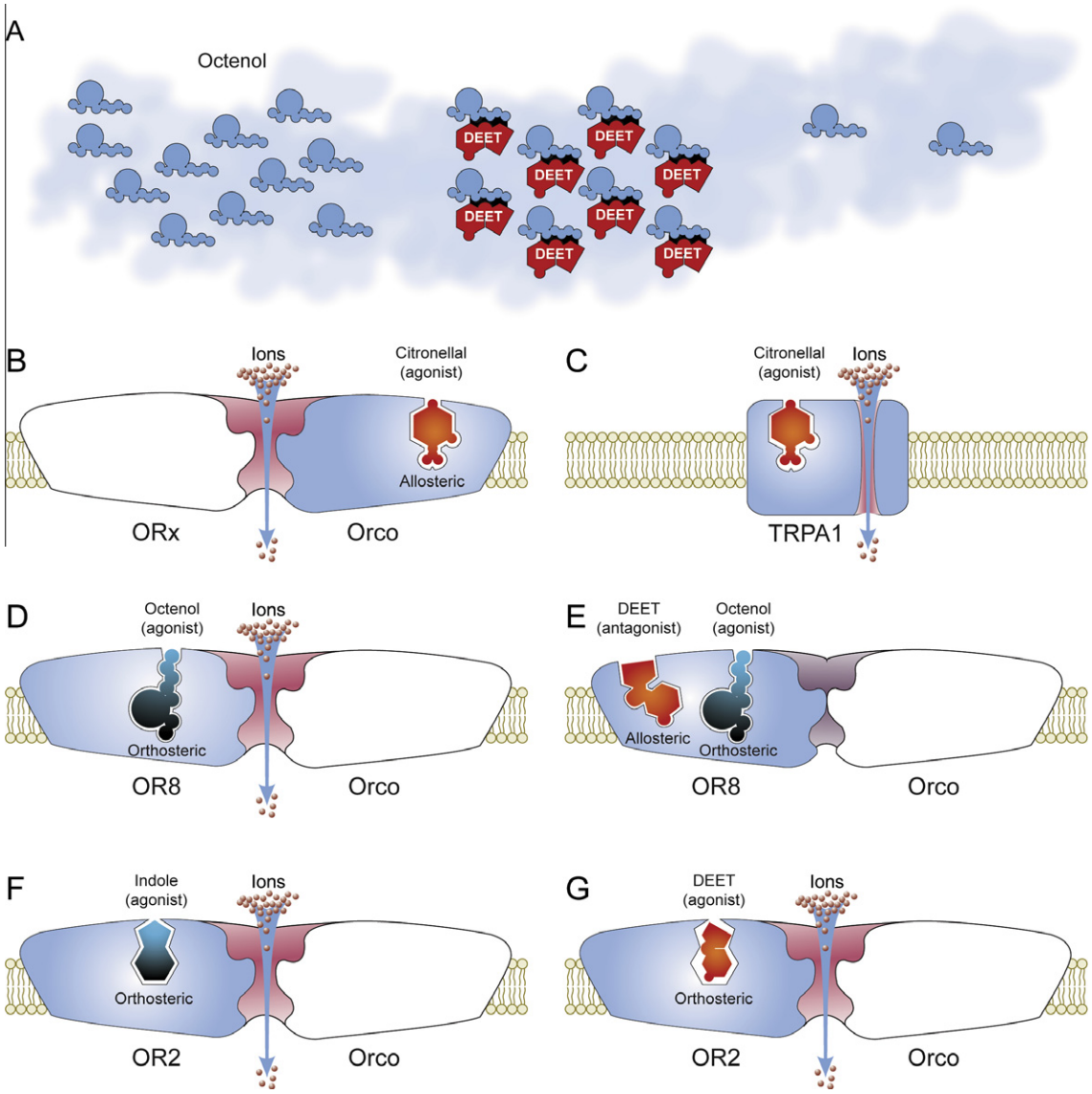

Fig. 1. Modes of action of insect repellents. A. Fixative effect of DEET on the attractant, octenol. B. Interaction of citronellal with a receptor assemblage through an allosteric site on *Drosophila* Orco. C. Activation of a mosquito TRPA1 channel by citronellal. D. Activation of OR8-Orco by interaction of octenol with the orthosteric site on OR8. E. Inhibition of octenol response by interaction of DEET with an allosteric site on OR8. F. Activation of OR2-Orco by interaction of indole with the orthosteric site on OR2. G. Activation of OR2-Orco by interaction of DEET with the orthosteric site on OR2.

amount of octenol released from the cartridge was reduced. This effect led to smaller responses of octenol ORNs in *Ae. aegypti*. They also showed that DEET applied to the skin changed the "chemical profile" of volatiles being released, perhaps decreasing the attractiveness of the skin. However, this fixative effect (Fig. 1A) was refuted by another study by Pellegrino et al. [10].

2.4. DEET stimulates a gustatory receptor neuron (GRN) sensitive to bitter aversive compounds in Drosophila

Lee et al. [11] showed that DEET suppresses the feeding behavior of the vinegar fly, *Drosophila melanogaster*. GRNs housed in the short sensilla on the outer labellum of the fly responded to both DEET and other bitter feeding deterrents such as quinine. These effects were determined to be mediated by direct interactions between DEET and several gustatory receptors (GRs).

2.5. A botanical repellent, citronellal, interacts with two distinct molecular pathways to mediate repellency

Kwon et al. [12] showed that citronellal interacted with the olfactory co-receptor Orco and with TRPA1 channels in *An. gambiae* and *D. melanogaster* (Fig. 1B and C). In *An. gambiae*, the TRPA1 channel is directly activated by citronellal, whereas in *Drosophila*, citronellal may regulate the activity of a Ca^{2+}-activated K^+ channel by interacting with TRPA1.

2.6. DEET modulates responses of specific ORNs and ORs to their ligands

Bohbot and Dickens [13] used *Xenopus* oocytes as an *ex vivo* expression system to explore the molecular receptive range of *Ae. aegypti* ORs. These pharmacological studies revealed that the activity of ORs could be modulated by a variety of insect repellents. This idea provided support for an earlier study that showed that DEET

Please cite this article in press as: J.C. Dickens, J.D. Bohbot, Mini review: Mode of action of mosquito repellents, Pestic. Biochem. Physiol. (2013), http://dx.doi.org/10.1016/j.pestbp.2013.02.006

and other repellents may stimulate specific ORs (Fig. 1D and E) or inhibit responses of ORs to attractants in *Ae. aegypti* (Fig. 1F and G). Thus, messages received by the CNS were scrambled resulting in disorientation of the insect.

2.7. Old insights stimulate new questions and answers

After the numerous efforts over 60 years since the discovery of DEET, the mode of action of DEET and other repellents remains a matter of debate. In the last 5 years a handful of reports have implicated olfactory, gustatory and temperature signaling pathways. While these studies have begun to uncover the mechanisms of action of insect repellents, they have also generated new questions. How can DEET and other repellents interact with unrelated receptor proteins? Does this question relate to the broad spectrum activity of repellents on phylogenetically diverse arthropods? How does the activation and inhibition of ORs by DEET relate to its effects on insect behavior? The following sections focus on the newer discoveries regarding the physiological and molecular effects of repellents on ORs and GRs in an attempt to address these intriguing questions.

3. Action of repellents on ORNs and ORs

3.1. Electrophysiological techniques used to study ligand-gated ORs

Electrophysiological recordings from ORNs *in vivo* and ORs expressed heterologously (=*ex vivo*) offer a powerful combination of tools for the study of the mode of action of mosquito repellents. The biochemical environment of ORs remains an active field of research as we have only a partial understanding of the components involved. Insect ORs are heteromeric ligand-gated ion channels. They are comprised of a variable odorant-sensing subunit (ORx) and an obligatory and invariable OR co-receptor (Orco) that form ORx-Orco [14–16]. While ORs play a pivotal role in olfactory signaling, other factors in the lymph of an olfactory sensillum, including odorant-binding proteins (OBPs), sensory neuron membrane proteins (SNMPs), and odorant-degrading enzymes (ODEs) may influence their function.

In order to eliminate these factors, ORs may be expressed outside the sensillum, in various cell expression systems such as the oocytes of the frog *Xenopus laevis*. This pharmacological technique allows for the establishment of precise concentration–response relationships, as the quantity of the stimulus to which the receptors are exposed is known. Prior knowledge of the OR and its natural ligand is strongly recommended. ORs, like other classes of receptors, are likely to possess multiple recognition sites including a putative primary "orthosteric" site interacting with evolutionary selected semiochemicals and secondary "allosteric" sites which react with chemicals with no specific biological meaning (e.g. synthetic compounds) [17].

In order to test the pharmacological information obtained using *Xenopus* oocytes, these same experiments may be conducted *in vivo* on basiconic sensilla, which house three ORNs [18] that can be functionally distinguished based on the shape and amplitude of their action potentials. The "A" neuron characterized by the largest amplitude action potential responds to CO_2 [19]. The "B" neuron has no known effective stimulus and constitutively generates medium sized action potentials. Finally, the "C" neuron, with the smallest amplitude action potential, responds to octenol [20] and is thought to express the OR8-Orco receptor assemblage [21]. While single-cell recording is a powerful technique to study neuron physiology *in vivo*, the establishment of dose–response relationships between ORN activity and odorants or drugs presents one notable limitation: the exact quantity of stimulus leaving the odor cartridge and reaching the surface of the ORN cannot be practically measured.

Octenol (=1-octen-3-ol) was identified as a natural attractant for mosquitoes in 1989 [22]. A study using single-cell recordings in *Culex pipiens* demonstrated the selectivity of the "C" neuron, housed in basiconic sensilla on the maxillary palps, toward the (*R*)-enantiomer of octenol [23]. The same year, two-electrode voltage clamp of *Xenopus* oocytes expressing the *An. gambiae* octenol receptor (OR8-Orco), revealed increased responses to the (*R*)-enantiomer of octenol [21] (Fig. 1D). This information, in combination with expression data [21,24], strongly suggested that the natural odorant ligand for OR8 was (*R*)-octenol and paved the way for a detailed pharmacological analysis with the *Ae. aegypti* OR8 ortholog [25].

3.2. The odorant receptive range of ORNs and ORs

Using voltage clamp recordings, OR8-Orco responses were evaluated when exposed to octenol and various analogs with minimal structural modifications (Fig. 2) [25]. OR8-Orco was estimated to be 100-fold more sensitive to the (*R*) enantiomer than to the (*S*) form, a difference likely underestimated considering the presence of trace amounts of the opposite enantiomer in each tested sample. Perhaps more remarkable was that OR8-Orco affinity was stronger for other octenol analogs than for (*S*)-octenol. This experiment demonstrated that an OR was able to achieve remarkable sensitivity and specificity toward a non-pheromonal compound.

Results of the *ex vivo* studies were tested *in vivo* using single-cell recordings from octenol sensitive ORNs in sensilla on the maxillary palps [26]. Using CO_2 free air, the "C" neuron was challenged with the same octenol analogs and the two octenol enantiomers tested in the heterologous expression system. While the actual concentration of odorant reaching the ORN could not be determined, the data concurred with those obtained from voltage clamp recordings from *Xenopus* oocytes expressing OR8-Orco. Increased differences in the sensitivity to (*R*)-octenol relative to other compounds observed *in vivo* were difficult to interpret due to inherent differences in stimulus delivery between voltage clamp and single-cell recording techniques. Thus, a role for accessory proteins such as OBPs could not be excluded in enhancing the sensitivity of OR8-Orco *in vivo*; many studies have suggested that OBPs might influence the solubility of odors within the sensillum lymph surrounding the neuron.

Two other ORs provided pharmacological prospects to study the effect of mosquito repellents. OR2 and OR10 are paralogous members of a conserved group of indole receptors present in both the aedine and anopheline mosquitoes [27]. Using voltage clamp recordings from *Xenopus* oocytes expressing OR2-Orco, indole was identified as a biologically meaningful ligand for OR2 (Fig. 1F). Due to the protein sequence identity between OR2 and OR10, it was predicted that the latter would specifically recognize an indole analog [27]. Subsequently, 3-methyl-indole (skatole) was shown to be the cognate ligand for OR10 [28]. These experiments led to three important conclusions: (1) ORs are capable of a remarkable level of discrimination without the assistance of other factors such as OBPs, (2) non-pheromone receptors exhibit ligand specificity and sensitivity on par with pheromone receptors, and (3) the molecular geometry of semiochemicals are a determinant factor for proper ligand recognition.

The results of the aforementioned studies agreed with the theory that ORs possess a highly specific primary recognition site (orthosteric site) for a biologically meaningful signal (semiochemical) and "specialist" ORs now included non-pheromonal receptors. These ideas required a re-examination of the concept of "generalist" ORs, i.e., receptors activated by ligands with various chemical geometry [17]. The ability of an OR to recognize a variety of ligands

Please cite this article in press as: J.C. Dickens, J.D. Bohbot, Mini review: Mode of action of mosquito repellents, Pestic. Biochem. Physiol. (2013), http://dx.doi.org/10.1016/j.pestbp.2013.02.006

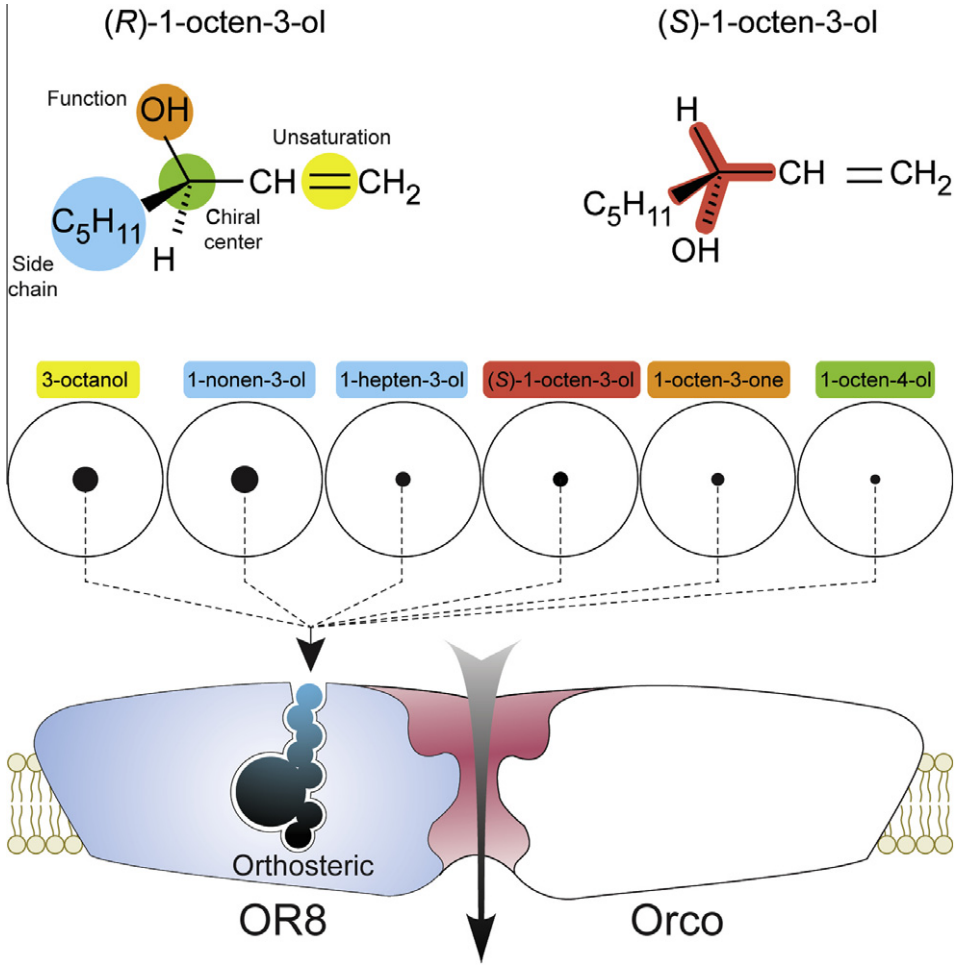

Fig. 2. Structure of (*R*)- and (*S*)-enantiomers of 1-octen-3-ol and analogs supposedly interacting with orthosteric site for (*R*)-1-octen-3-ol on OR8. Both octenol enantiomers and each of the compounds shown were tested on octenol receptor neurons *in vivo* and OR8-Orco expressed heterologously in *Xenopus* oocytes. The relative sensitivities of OR8-Orco toward (*R*)-1-octen-3-ol (○) and octenol analogs (●) were translated from EC_{50} values into surface areas.

requires either a single but variable-geometry ligand recognition site (single binding domain) or multiple recognition sites (multiple binding domains). Growing experimental evidence support the latter idea without excluding some limited degree of ligand promiscuity [25].

3.3. DEET modulates the activity of ORs

Separate studies have shown that DEET, a known insect repellent, selectively activates [9] or inhibits ORs [29]. This synthetic compound, not encountered in nature by insects, was nonetheless able to interact with ORs, albeit at high concentrations. How could this compound exert agonist and antagonist effects on different ORs? We used our knowledge of OR-ligand pairs to study this question. OR2, OR8 and OR10 exhibit a wide range of sequence similarities [24]. OR2 and OR10 share 69% amino acid identity, and 14% identity with OR8. These protein sequence variations provided a control to test the molecular basis of the possible mode of action of insect repellents. We also expanded our studies to include structurally different insect repellents including both synthetic compounds and naturally occurring chemicals [30] using *Xenopus* oocytes heterologously expressing OR2, OR8 or OR10 along with Orco.

These studies suggested that mosquito repellents exert multiple effects on ORs. For example, while DEET alone activated OR2-Orco (Fig. 1G), it inhibited OR8-Orco response to octenol [13] (Fig. 1E). DEET reduced OR sensitivity and the maximum effect of the agonist odorants [31]. A survey of the potential effects of various insect repellents on OR8-Orco and OR2-Orco confirmed that these compounds either elicited specific, dominant or unspecific agonist or antagonist effects depending on the ORx tested [30]. More importantly, these results were largely corroborated by *in vivo* studies showing that DEET altered the fine-tuning of functionally diverse ORNs [10]. However, the exact operating mechanisms for the mode of action of insect repellents, via orthosteric or allosteric sites, were not conclusively demonstrated and the multimeric nature of ORs compounded the interpretations of these results.

Using high-throughput screens to search for novel modulators of *An. gambiae* ORs expressed in human embryonic kidney cell lines, a new class of synthetic chemicals, known as VUAA were shown to activate Orco alone [31]. These results were the proof of concept for the existence of allosteric sites considering that Orco is the non-sensing subunit of the ORx-Orco complex. An additional study on the activation of Orco by a VUAA analog was consistent with the idea that Orco forms multimeric assemblages [32].

4. Action of repellents on GRNs and GRs

While mosquito repellents have mostly been studied in an olfactory context, several reports have indicated that repellents may be detected by via gustatory receptors (=contact chemoreceptors) on the labella (Fig. 3A and B) and function as feeding

Please cite this article in press as: J.C. Dickens, J.D. Bohbot, Mini review: Mode of action of mosquito repellents, Pestic. Biochem. Physiol. (2013), http://dx.doi.org/10.1016/j.pestbp.2013.02.006

deterrents. Christophers [33] suggested that compounds with low volatility acted as contact repellents. Bar-Zeev and Schmidt [34] used a radiotracer to present evidence suggesting that low concentrations of DEET were detected by contact chemoreceptors on the labella of *Ae. aegypti*. More recently DEET and other repellents were shown to act as feeding deterrents in behavioral bioassays [35,36]. The suggestion that DEET and other repellents acted as feeding deterrents indicates that gustatory receptors might be targeted by insect repellents.

We have recently used single-cell recordings from sensilla on the labella of *Ae. aegypti* to demonstrate the presence of a GRN that responds to DEET and other repellents including Picaridin, IR3535, and citronellal [37]. Based on the size and shape of the action potentials recorded, at least three GRNs are housed within individual sensilla on the labella (Fig. 3B). The neuron with the largest amplitude action potential responded to increasing concentrations of NaCl (Fig. 3C). A somewhat smaller amplitude action potential was activated by sucrose (Fig. 3D), while the smallest amplitude action potential was activated by the feeding deterrent, quinine (Fig. 3E). This neuron with the smallest amplitude action potential was also activated in a dose dependent manner by DEET, and responses were elicited by Picaridin, IR3535, and citronellal (Fig. 3E). GRNs in the fruit fly, *D. melanogaster* are also activated by DEET [11].

The discovery of a GRN that responds reliably to DEET and other repellents is a first for mosquitoes or, for that matter, any

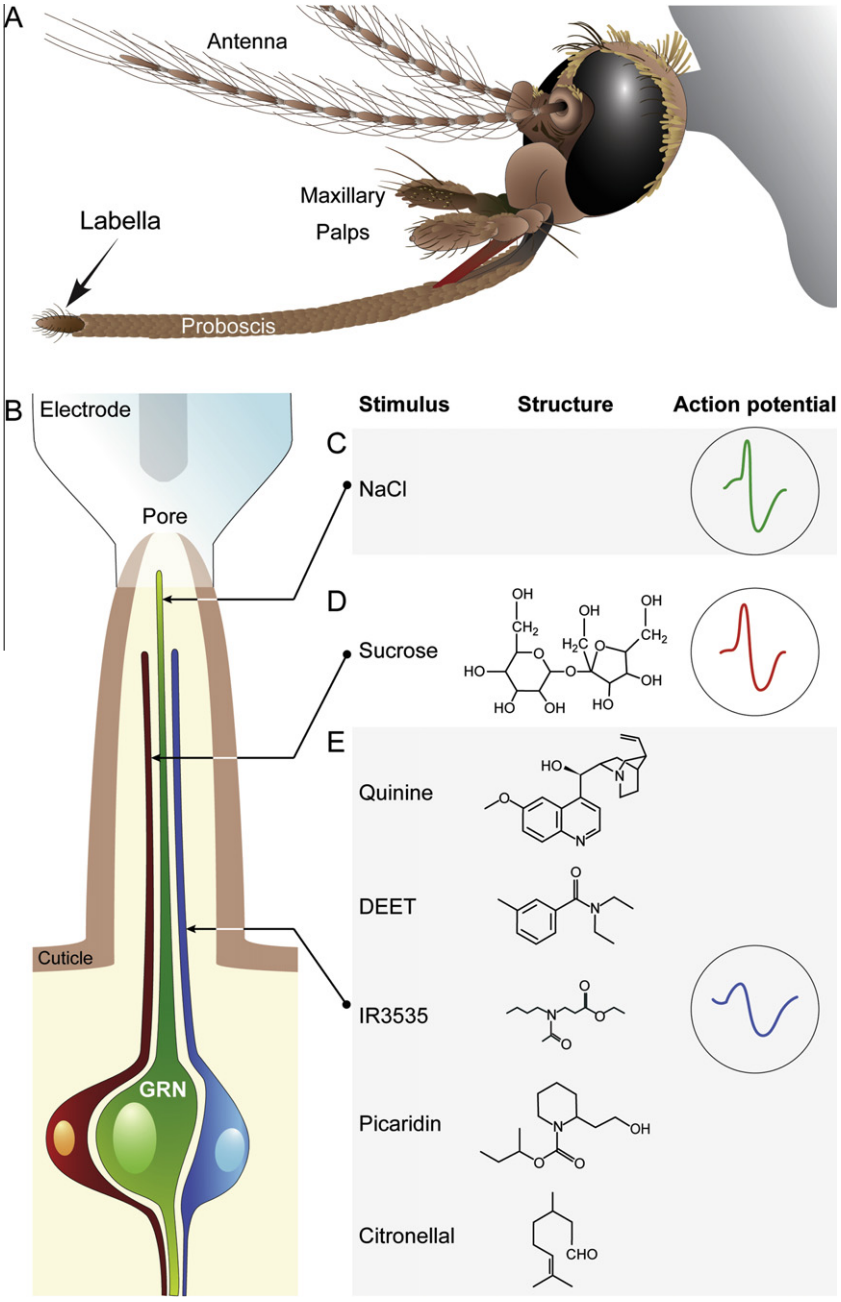

Fig. 3. A. Location of sensilla on the labella of *Ae. aegypti*. B. Electrophysiological recordings from sensilla on the labella revealed action potentials from at least 3 gustatory receptor neurons (GRNs). C. The largest amplitude action potential responded to increasing concentrations of NaCl. D. A somewhat smaller amplitude action potential responded to sucrose. E. The smallest amplitude action potential was activated by quinine, a feeding deterrent, and the insect repellents DEET, IR3535, Picaridin, and citronellal.

Please cite this article in press as: J.C. Dickens, J.D. Bohbot, Mini review: Mode of action of mosquito repellents, Pestic. Biochem. Physiol. (2013), http://dx.doi.org/10.1016/j.pestbp.2013.02.006

hematophagous arthropod. This deterrent GRN resembles other GRNs found in herbivorous insects that mediate avoidance behaviors [38]. The fact that the neuron was sensitive for the repellents tested may provide, in part, an alternative explanation for the requirement for high concentrations of these repellents for their effects as GRNs in other insects including the blowfly, *Phormia regina*, respond to volatiles at high concentrations [39]. Since DEET, Picaridin, and IR3535 are synthetic compounds, and citronellal differ structurally from each other (Fig. 3E), it seems likely that some repellents interact with allosteric sites on the GR assemblage as better demonstrated for the olfactory sense [29,40].

5. Conclusions

Insect repellents exert their effects through interactions with ORs and GRs in mosquitoes. The fact that DEET modulates responses of ORs *ex vivo* in *Ae. aegypti* [13] is supported by studies in *Drosophila* where DEET was shown to alter responses of ORNs to their ligands based on the concentration of the odor [10]. In addition, DEET, IR3535, Picaridin, and citronellal stimulate a specific GRN sensitive to feeding deterrents in *Ae. aegypti* [37].

How do DEET and other insect repellents interact with a multiplicity of transmembrane proteins engaged in various sensory modalities? It appears that part of the answer lies with the ability of these compounds to interact with secondary recognition sites (allosteric sites) on receptor proteins. It is also important to note that these effects are observed when using high quantities of insect repellents. These characteristics may explain how DEET affects the behavior of diverse groups of arthropods. However, the receptor specific effects of some repellents indicate that these compounds have structural qualities that enable interactions with a variety of molecular targets. This question will be best tested by looking at the molecular mode of action of DEET on non-insect arthropods such as ticks.

Pioneering independent studies had led to distinct theories on the molecular mode of action of DEET in olfaction: (i) DEET alone activates ORx-Orco in the absence of odorant or (ii) that DEET inhibits the activation of ORx-Orco by the cognate ligand. We have shown that both theories are not exclusive and depend on the ORx-Orco construct and ligand contexts (presence or absence of cognate ligands).

Understanding the mode of action of insect repellents and how these chemicals interact with odorants to modulate OR and GR activity will allow us to design potent formulations aimed at interfering with insect sensory signaling to ultimately disrupt their cognitive processes. It is also important to underscore the need to understand the chemical ecology of mosquitoes and other targeted pests in order to provide candidate semiochemicals necessary to develop better attractants and repellent formulations. Identification of new synthetic repellents or compounds with novel modes of action will be facilitated by heterologous expression of targeted ORs and GRs for discovery of allosteric recognition sites.

Acknowledgments

The authors are grateful for reviews of the manuscript by Dr. Daniel Strickman, USDA, ARS, Beltsville, MD and Dr. Jackson Sparks, University of South Carolina. This work was supported in part by a grant to JCD from the Deployed War Fighter Protection (DWFP) Research Program.

References

[1] M. Debboun, D. Strickman, Insect repellents and associated personal protection for a reduction in human disease, Med. Vet. Entomol. 27 (2013) 1–9.
[2] M. Brown, A.A. Hebert, Insect repellents: an overview, J. Am. Acad. Dermatol. 36 (1997) 243–249.
[3] M. Debboun, S.P. Frances, D. Strickman, Insect Repellents: Principles, Methods, and Uses, CRC Press, Boca Raton, FL, 2007.
[4] E.E. Davis, Insect repellents: concepts of their mode of action relative to potential sensory mechanisms in mosquitoes (Diptera: Culicidae), J. Med. Entomol. 22 (1985) 237–243.
[5] E.E. Davis, P.G. Sokolove, Lactic acid-sensitive receptors on the antennae of the mosquito, *Aedes aegypti*, J. Comp. Physiol. A 105 (1976) 43–54.
[6] A. Kuthiala, R.K. Gupta, E.E. Davis, Effect of the repellent deet on the antennal chemoreceptors for oviposition in *Aedes aegypti* (Diptera: Culicidae), J. Med. Entomol. 29 (1992) 639–643.
[7] J. Boeckh, H. Breer, M.M. Geier, F. Hoever, B. Kruger, G. Nentwig, H. Sass, Acylated 1,3-aminopropanols as repellents against bloodsucking arthropods, Pest. Sci. 48 (1996) 359–373.
[8] Z. Syed, W.S. Leal, Mosquitoes smell and avoid the insect repellent DEET, Proc. Natl. Acad. Sci. USA 105 (2008) 13598–13603.
[9] Y. Xia, G. Wang, D. Buscariollo, J. Pitts, R.H. Wenger, L. Zwiebel, The molecular basis of olfactory-based behavior in *Anopheles gambiae* larvae, Proc. Natl. Acad. Sci. USA 105 (2008) 6433–6438.
[10] M. Pellegrino, N. Steinbach, M.C. Stensmyr, B.S. Hansson, L.B. Vosshall, A natural polymorphism alters odour and DEET sensitivity in an insect odorant receptor, Nature 478 (2011) 511–514.
[11] Y. Lee, S.H. Kim, C. Montell, Avoiding DEET through insect gustatory receptors, Neuron 67 (2010) 555–561.
[12] Y. Kwon, S.H. Kim, D.S. Ronderos, Y. Lee, B. Akitake, O.M. Woodward, W.B. Guggino, D.P. Smith, C. Montell, *Drosophila* TRPA1 channel is required to avoid the naturally occurring insect repellent citronellal, Curr. Biol. 20 (2010) 1672–1678.
[13] J.D. Bohbot, J.C. Dickens, Insect repellents: modulators of mosquito odorant receptor activity, PLoS ONE 5 (2010) e12138.
[14] T. Nakagawa, T. Sakurai, T. Nishioka, K. Touhara, Insect sex-pheromone signals mediated by specific combinations of olfactory receptors, Science 307 (2005) 1638–1642.
[15] K. Sato, M. Pellegrino, T. Nakagawa, T. Nakagawa, L.B. Vosshall, K. Touhara, Insect olfactory receptors are heteromeric ligand-gated ion channels, Nature 452 (2008) 1002–1006.
[16] D. Wicher, R. Schafer, R. Bauernfeind, M.C. Stensmyr, R. Heller, S.H. Heinemann, B.S. Hansson, *Drosophila* odorant receptors are both ligand-gated and cyclic-nucleotide-activated cation channels, Nature 452 (2008) 1007–1011.
[17] J.D. Bohbot, J.C. Dickens, Selectivity of odorant receptors in insects, Front. Cell. Neurosci. 6 (2012) 29.
[18] S.B. McIver, Fine structure of pegs on the palps of female culicine mosquitoes, Can. J. Zool. 50 (1972) 571–576.
[19] A.J. Grant, B.E. Wigton, J.G. Aghajanian, R.J. O'Connell, Electrophysiological responses of receptor neurons in mosquito maxillary palp sensilla to carbon dioxide, J. Comp. Physiol. A 177 (1995) 389–396.
[20] A. Grant, R. O'Connell, Electrophysiological responses from receptor neurons in mosquito maxillary palp sensilla, in: G.R. Block, G. Cardew (Eds.), Olfaction in Mosquito-host interactions, Wiley, Chichester, UK, 1996.
[21] T. Lu, Y.T. Qiu, G. Wang, J.Y. Kwon, M. Rutzler, H.W. Kwon, R.J. Pitts, J.J. Van Loon, W. Takken, J.R. Carlson, L.J. Zwiebel, Odor coding in the maxillary palp of the malaria vector mosquito *Anopheles gambiae*, Curr. Biol. 17 (2007) 1533–1544.
[22] W. Takken, D.L. Kline, Carbon dioxide and 1-octen-3-ol as mosquito attractants, J. Am. Mosq. Control Assoc. 5 (1989) 311–316.
[23] Z. Syed, W.S. Leal, Maxillary palps are broad spectrum odorant detectors in *Culex quinquefasciatus*, Chem. Senses 32 (2007) 727–738.
[24] J. Bohbot, R.J. Pitts, H.W. Kwon, M. Rutzler, H.M. Robertson, L.J. Zwiebel, Molecular characterization of the *Aedes aegypti* odorant receptor gene family, Insect Mol. Biol. 16 (2007) 525–537.
[25] J.D. Bohbot, J.C. Dickens, Characterization of an enantioselective odorant receptor in the yellow fever mosquito *Aedes aegypti*, PLoS ONE 4 (2009) e7032.
[26] A.J. Grant, J.C. Dickens, Functional characterization of the octenol receptor neuron on the maxillary palps of the yellow fever mosquito, *Aedes aegypti*, PLoS ONE 6 (2011) e21785.
[27] J.D. Bohbot, P.L. Jones, G. Wang, R.J. Pitts, G.M. Pask, L.J. Zwiebel, Conservation of indole responsive odorant receptors in mosquitoes reveals an ancient olfactory trait, Chem. Senses 36 (2010) 149–160.
[28] D.T. Hughes, J. Pelletier, C.W. Luetje, W.S. Leal, Odorant receptor from the southern house mosquito narrowly tuned to the oviposition attractant skatole, J. Chem. Ecol. 36 (2010) 797–800.
[29] M. Ditzen, M. Pellegrino, L.B. Vosshall, Insect odorant receptors are molecular targets of the insect repellent DEET, Science 319 (2008) 1838–1842.
[30] J.D. Bohbot, L. Fu, T.C. Le, K.R. Chauhan, C.L. Cantrell, J.C. Dickens, Multiple activities of insect repellents on odorant receptors in mosquitoes, Med. Vet. Entomol. 25 (2011) 436–444.
[31] P.L. Jones, G.M. Pask, D.C. Rinker, L.J. Zwiebel, Functional agonism of insect odorant receptor ion channels, Proc. Natl. Acad. Sci. USA 108 (2011) 8821–8825.
[32] J.D. Bohbot, J.C. Dickens, Odorant receptor modulation: ternary paradigm for mode of action of insect repellents, Neuropharmacology 62 (2012) 2086–2095.
[33] S.R. Christophers, Mosquito repellents: being a report of the work of the mosquito repellent inquiry, Cambridge 1943–5, J. Hyg. 45 (1947) 176–231.

Please cite this article in press as: J.C. Dickens, J.D. Bohbot, Mini review: Mode of action of mosquito repellents, Pestic. Biochem. Physiol. (2013), http://dx.doi.org/10.1016/j.pestbp.2013.02.006

[34] M. Bar-Zeev, C.H. Schmidt, Action of a repellent as indicated by a radioactive tracer, J. Econ. Entomol. 52 (1959) 268–269.
[35] J.A. Klun, A. Khrimian, M. Debboun, Repellent and deterrent effects of SS220, Picaridin, and DEET suppress human blood feeding by *Aedes aegypti*, *Anopheles stephensi*, and *Phlebotomus papatasi*, J. Med. Entomol. 43 (2006) 34–39.
[36] K.R. Chauhan, J.A. Klun, M. Debboun, M. Kramer, Feeding deterrent effects of catnip oil components compared with two synthetic amides against *Aedes aegypti*, J. Med. Entomol. 42 (2005) 643–646.
[37] J.L. Sanford, V.D.C. Shields, J.C. Dickens, Gustatory receptor neuron responds to DEET and other insect repellents in the yellow fever mosquito, *Aedes aegypti*. *Naturwissenschaften*, 2013, http://dx.doi.org/10.1007/s00114-013-1021-x.
[38] L.M. Schoonhoven, J.J.A. van Loon, An inventory of taste in caterpillars: each species its own key, Acta Zool. Acad. Sci. Hung. 48 (2002) 215–263.
[39] V.G. Dethier, Sensitivity of the contact chemoreceptors of the blowfly to vapors, Proc. Natl. Acad. Sci. USA 69 (1972) 2189–2192.
[40] B.W. Bissinger, C.S. Apperson, D.E. Sonenshine, D.W. Watson, R.M. Roe, Efficacy of the new repellent BioUD against three species of ixodid ticks, Exp. Appl. Acarol. 48 (2009) 239–250.

Please cite this article in press as: J.C. Dickens, J.D. Bohbot, Mini review: Mode of action of mosquito repellents, Pestic. Biochem. Physiol. (2013), http://dx.doi.org/10.1016/j.pestbp.2013.02.006